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MORPHOLOGY OF THE CORTICAL REACTION IN THE EGGS OF *PENAEUS AZTECUS*

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Egg cortical reactions initiated by sperm penetration or exposure to sea water have long been known in many animal ova. Cortical reactions exist in the eggs of cnidarians (Dewel and Clark, 1974), annelids (Pasteels, 1965), arthropods (Hudinaga, 1942), echinoderms (Afzelius, 1956), teleosts (Yamamoto, 1939; Kagan, 1935), amphibians (Osanai, 1960), and mammals (Austin and Amoroso, 1959; Szollosi, 1962; Austin, 1965 and 1968, for review).

Dehiscence of the cortical granules at the periphery of a typical ovum into the surrounding media often causes the lifting of an egg investment coat with the resultant formation of a fertilization membrane (Endo, 1961a, b). It is believed that these cortical reactions are responsible for the prevention of polyspermy by both a chemical and physical block (Epel, 1975 for review) and that they also may establish a microenvironment inside a tough chorionic membrane for the developing embryo (Allen, 1958).

Changes in the cortices of animal ova have been studied extensively in the echinoderms, annelids, and amphibians. At present, however, there is very little information concerning egg activation in crustaceans. Since decapod crustacean fertilization is often internal, it is difficult to observe reactions of the egg. Hinsch (1971) reported the formation of a membrane around the eggs of *Libinia emarginata* that might be the result of a cortical reaction. Cheung (1966) has reported membrane formation probably initiated by fertilization around the eggs of *Carcinus*.

Work on the ovaries and the cortical structure in eggs of animals of the natantia is limited. Hudinaga (1942) briefly described a massive cortical reaction in the eggs of *Penaeus japonicus* and *P. monoceros*. In both animals, regions of "jellylike substance" were described in the peripheral cytoplasm of the eggs. These peripheral regions were released at spawning and formed a jelly coating around the surface of the egg that remained until the second cleavage.

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Preliminary reports (Clark and Lynn, 1977; Lynn and Clark, 1975; Clark, Lynn and Yudin, 1975) have described the cortical reaction in the eggs of *P. aztecus* and *P. setiferus*. The purpose of this paper is to carefully document the morphological events that occur in the cortex of *Penaeus* sp. ova upon contact with sea water.

Materials and Methods

Using a standard otter trawl, gravid brown shrimp (*P. aztecus*) were collected 80 to 100 miles south of Galveston, Texas, and gravid white shrimp (*P. setiferus*) were caught 1 to 10 miles off the coast. Observations reported here, including figures, concern *P. aztecus*. Findings were confirmed with *P. setiferus*.

Animals were transported to the laboratory in a 150-gal aerated tank held at 15 to 20° C. Once in the laboratory, the gravid shrimp were placed in aerated, inverted, 5-gal carboys, and the water temperature was raised slowly to 28° C to induce spawning.

For transmission electron microscopy, eggs were fixed for 1 to 2 hr in a 0.1 M, PO_4-buffered (pH 7.5) paraformaldehyde-glutaraldehyde solution, with sucrose added to adjust osmolarity (Karnovsky, 1965). Samples then were post-fixed in 0.1 M, PO_4-buffered osmium tetroxide for 30 min. Fixed samples were dehydrated rapidly in a graded acetone series, embedded in a low-viscosity epoxy resin (Spurr, 1969), and sectioned with either glass or diamond knives on a Porter Blum MT2-B ultramicrotome. Thick plastic sections for light microscopy were stained with 1% toluidine blue buffered in 0.15% sodium borate (Dewel and Clark, 1974). Thin sections were stained with saturated methanolic uranyl acetate, counterstained with lead citrate (Venable and Coggeshall, 1965), and examined on a Hitachi HS-8 electron microscope.

For scanning electron microscopy, eggs prior to and at various stages of activation were fixed in a paraformaldehyde-glutaraldehyde solution as previously described, dehydrated in a graded acetone series, and critical-point dried. Samples then were coated with approximately 20 nm of gold and examined on a Cambridge S4-10 Stereoscan microscope.

Results

Light microscopy of the cortical reaction

A mature, unreacted oocyte spawned directly into fixative is spherical and approximately 266 μm in diameter (Fig. 1A). Light striations, which represent cortical specializations (cortical rods), are apparent in the peripheral cytoplasm. When an oocyte is spawned into sea water, these cortical rods are released into the surrounding media. Figures 1A to 1L represent a series of light micrographs illustrating this reaction.

Early stages of the reaction are stopped for observation (Figs. 1B, 1C) by holding a spawning female over a beaker of sea water and fixing the collected eggs at 10 and 20 seconds, respectively. In these micrographs (1B, 1C), the rods have just begun to emerge from the egg and result in a blebbed appearance around the periphery of the egg. Figures 1D to 1L illustrate one egg undergoing a cortical reaction.

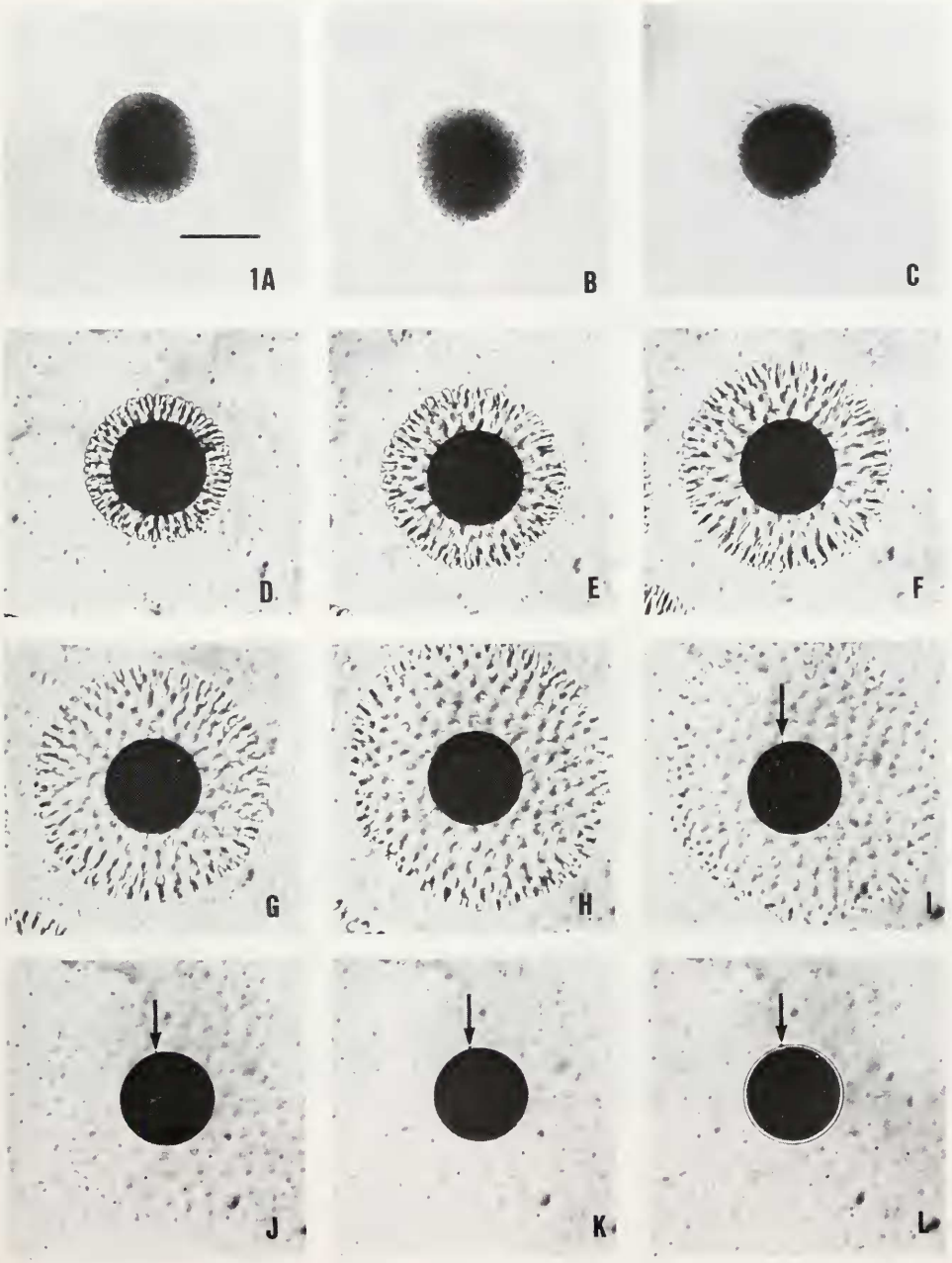


FIGURE 1A–1L. Light micrographs showing the cortical reaction of *P. aztecus* eggs from the unreacted stage, through the release of the polar body (arrow), to the formation of a hatching membrane. Bar = 200 μm.

Once the cortical rods are expelled, they form a corona around the egg (Fig. 1E). This corona remains as the rods swell and begin to dissipate (Figs. 1G, 1J). Within 5 to 7 min after initiation of the reaction, the cortical rods

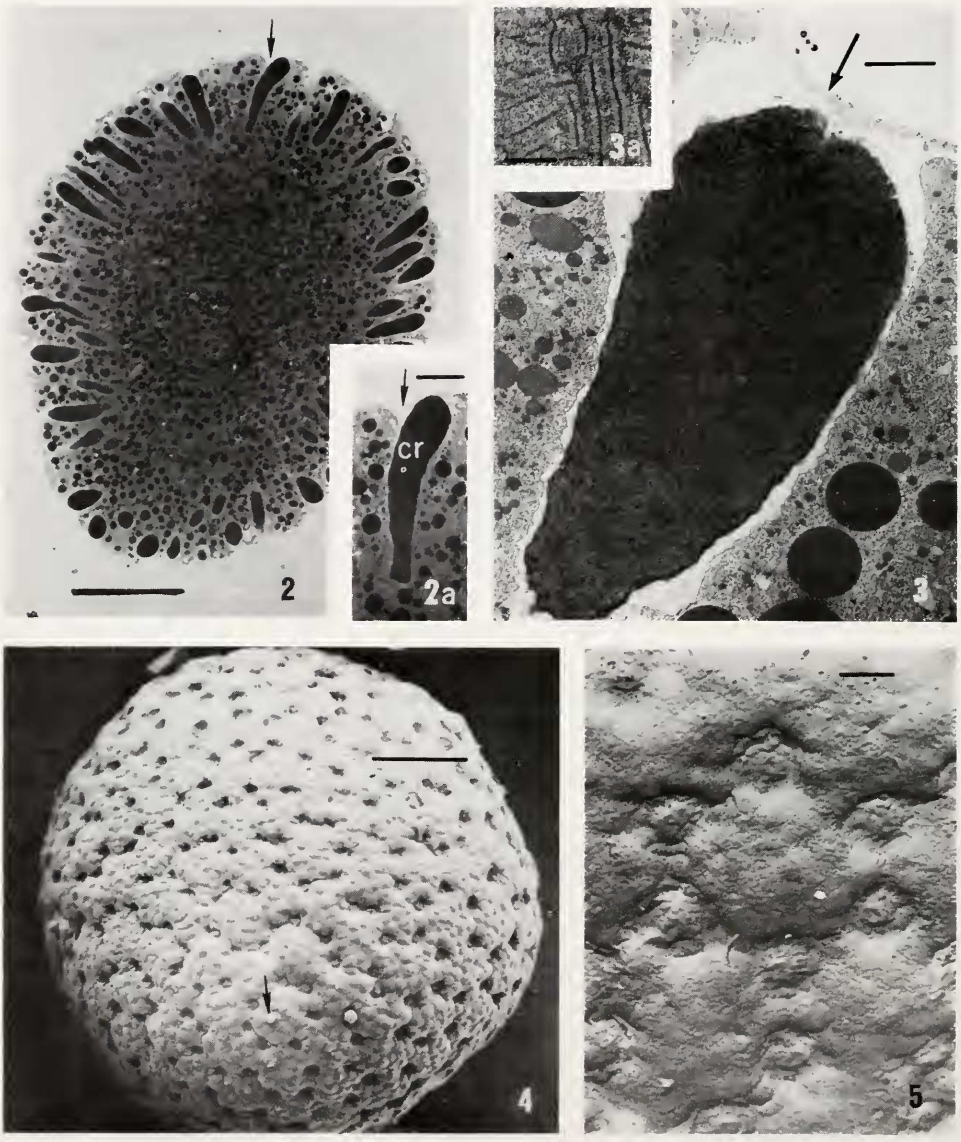


FIGURE 2. A light micrograph of a thick plastic section of an unreacted oocyte. Arrow points to the investment coat. Bar = 50 μm.

FIGURE 2a. A higher magnification showing the investment coat (arrow), which separates the club-shaped cortical rod (CR) from the external media. Bar = 10 μm.

FIGURE 3. A transmission electron micrograph of a cortical rod (CR) within its crypt. The investment coat is indicated by arrows. Bar = 2 μm.

FIGURE 3a. A high magnification revealing the fibrillar substructure of the cortical rods. Bar = 0.2 μm.

FIGURE 4. Scanning electron micrograph of an unreacted oocyte. Indentations in the investment coat represent the location of the cortical rods' crypts. A sperm attached to the investment coat is also shown (arrow). Bar = 50 μm.

FIGURE 5. A higher magnification revealing the indentations arranged hexagonally on the surface of the egg. Bar = 5 μm.

are dissipated completely (Fig. 1K). After cortical rod dissipation, a hatching membrane begins to form and is readily apparent after 15 min (Fig. 1L).

To facilitate a complete morphological description of the above events, the cortical reaction is divided into four stages. These stages are: unreacted stage; early reaction; corona stage; and dissipation stage. The formation of the hatching membrane is not discussed in this paper.

Unreacted stage

As noted previously, to examine unreacted oocytes, the eggs were spawned directly into fixative. The oocytes contain extensive accumulations of yolk platelets dispersed throughout the cytoplasm (Fig. 2). Large, conspicuous, "club"-shaped cortical rods are located in the peripheral cytoplasm (Figs. 2, 3). Each cortical rod is approximately 39.9-μm long with a basal diameter of 4.6 μm and an apical diameter of 9.3 μm (Fig. 2A). The rods lie within membrane crypts and are separated from the external media by a thin fibrous investment coat, 0.13-μm thick, that encompasses the egg (Figs. 2, 3). The substructure of the rods is distinct, each rod being composed of numerous, tightly packed, feathery elements (Fig. 3A). Each element is composed of a dense axis, 9-nm wide and of variable length, with lateral fibrillar projections that are 3.5 nm wide and 31.5 nm long (Fig. 3A). The overall structure of each element is reminiscent of a "bottle brush." When unreacted oocytes are viewed with the scanning electron microscope (SEM), hexagonal patterns of cortical rod crypts are revealed as indentations in the investment coat (Figs. 4, 5). Occasional sperm also are noted on the surface of the investment coat (Fig. 4).

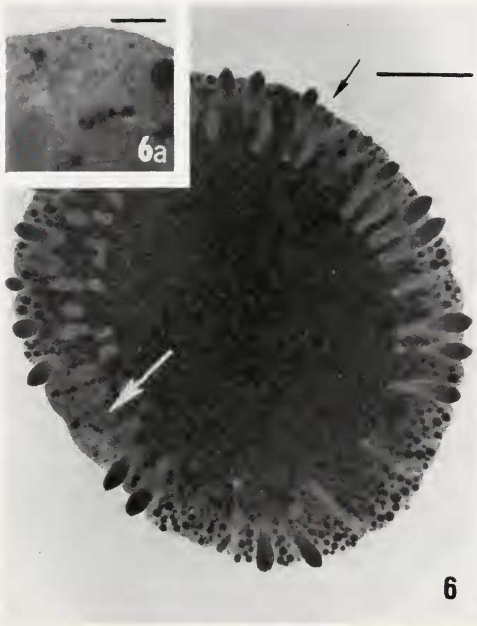
An unreacted oocyte is approximately 266-μm in diameter, or 9.85×10^6 μm^3 in volume, with a surface area of 2.2×10^5 μm^2. An oocyte has 746 ± 60 crypts. Assuming that each crypt is conically shaped, 39.9 μm long, and with an apical diameter of 9.3 μm and a basal diameter of 4.6 μm, the approximate volume and membrane surface area of each crypt can be determined (Table I).

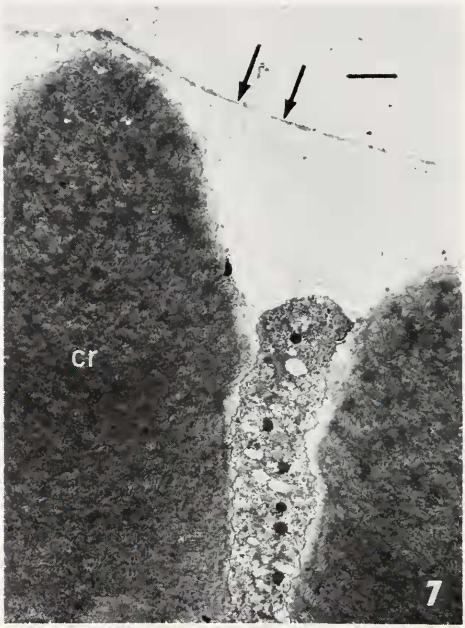
Using this approach, the crypts in any one oocyte account for a volume of 1.17×10^6 μm^3, or 12% of the oocyte's total volume, and a membrane surface area of 6.63×10^5 μm^2. Since the crypt membranes are continuous with the oolemma, the oocyte has an oolemma surface area of 8.35×10^5 μm^2, of which 79.5% is contained in the crypts.

TABLE I

Egg and rod size

Unit	Sample size	Unactivated eggs	Activated eggs
Egg diameter	20	266 ± 13 μm	237 ± 10 μm
Rods per egg	10	746 ± 60	
Rod size			
Length	20	39.9 ± 1.5 μm	
Diameter			
Top	20	9.3 ± 2.3 μm	
Bottom	20	4.6 ± 1.3 μm	





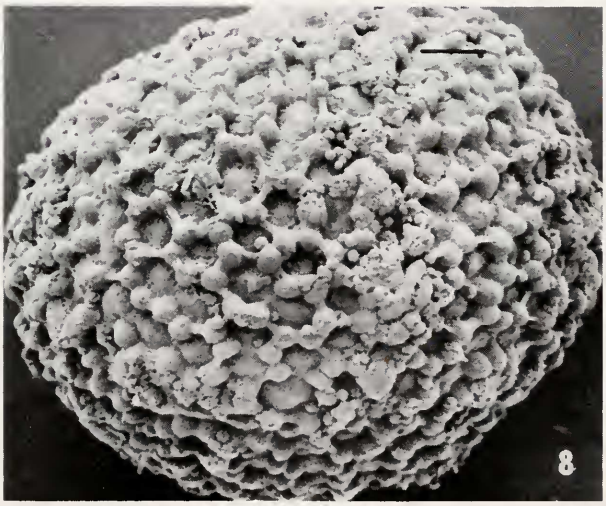


FIGURE 6. A light micrograph illustrating an oocyte at the beginning of its cortical reaction. The lifting of the investment coat (black arrow) and the appearance of a meiotic figure (white arrow) are shown. Bar = 50 μm.

FIGURE 6a. The first meiotic metaphase. Bar = 10 μm.

FIGURE 7. An electron micrograph of cortical rods emerging and lifting the investment coat off the egg. Bar = 1 μm.

FIGURE 8. A scanning electron micrograph showing early stages of the cortical reaction. Bar = 30 μm.

FIGURE 9. The emerging rods are clearly shown lifting the investment coat (arrows) and a few sperm(s) from the egg surface. Bar = 10 μm.

Early reaction

At this stage of the reaction, the rods slightly protrude from the surface of the oocyte (Figs. 6, 7), creating the blebbed appearance previously noted with phase

microscopy. While there are no apparent changes in the substructure of the rods, their protrusion coincides with the lifting of the investment coat from the egg surface. When viewed with SEM, the emerging cortical rods appear as prominent blebs in the investment coat (Fig. 8). At a higher magnification, the investment coat is seen stretched between emerging rods and undergoing a partial loss of integrity (Fig. 9). Sperm still are seen closely associated with the egg surface (Fig. 9).

The first meiotic division becomes apparent during activation, as evidenced by metaphase figures in the peripheral cytoplasm of the oocytes (Figs. 6, 6a).

Corona stage

By this stage of activation, the cortical rods are well out of their crypts (Figs. 10, 11, 12). There are still no apparent changes in the substructure of the rods, though membranous vesicles are noted between the rod and crypt membranes as well as along the outside of the oolemma (Fig. 11). The integrity of the investment coat is lost almost entirely, and when viewed with the SEM, the coat appears only as fragmented strands stretched between cortical rods (Fig. 12.). Elimination of the investment coat reveals small depressions in the surface of the oolemma as well as numerous sperm still associated with the surface of the egg at this stage of development (Fig. 12). Transmission microscopy clearly shows these sperm, which apparently have lost their terminal spikes and undergone activation (Clark, Talbot, Neal, Mock and Salser, 1974), in alliance with the egg oolemma (Fig. 13). An electron-dense material always is noted along the periphery of the activated surface of the sperm (Fig. 13) as well as in association with the opposed gamete membranes (Fig. 14).

Dissipation stage

As the cortical rods are released from their crypts, they undergo notable changes. There is a distinct swelling at both their basal and apical ends accompanied by a loss of substructural integrity in these regions (Figs. 16, 17). The "bottle brush" structures appear to break down, resulting in a flocculent material that eventually surrounds the egg (Fig. 17). During this period of rod dissipation, membranous vesicles, as noted earlier, become more apparent (Figs. 17, 18, 19). These vesicular elements, which are initially associated with the crypts, become dispersed around the entire egg surface (Figs. 18, 19). Eggs, initially 266 μm in diameter, measure 237 μm in diameter once the cortical reaction is completed.

Discussion

The cortical reaction typical in the eggs of penaeid shrimp is unique with respect to the absence of membrane fusion during the reaction, the size of the cortical specializations, and the rapid expulsion and dissipation of these specializations in response to sea water. Few examples of such a massive cortical reaction are reported in the literature. A morphologically similar reaction occurs in the oocytes of *Nereis limbata* (Novikoff, 1939; Costello, 1949; Fallon and Austin, 1967). Fibrous whorls of large "alveolar vesicles" form a broad cytoplasmic band at the cortex of *Nereis* eggs. This alveolar material is released at fertilization and forms a jellylike layer around the egg that remains throughout early development of the zygote. The fibrous nature of the cortical alveolae is similar to the cortical rods; however, the alveolae lack the feathery substructure.

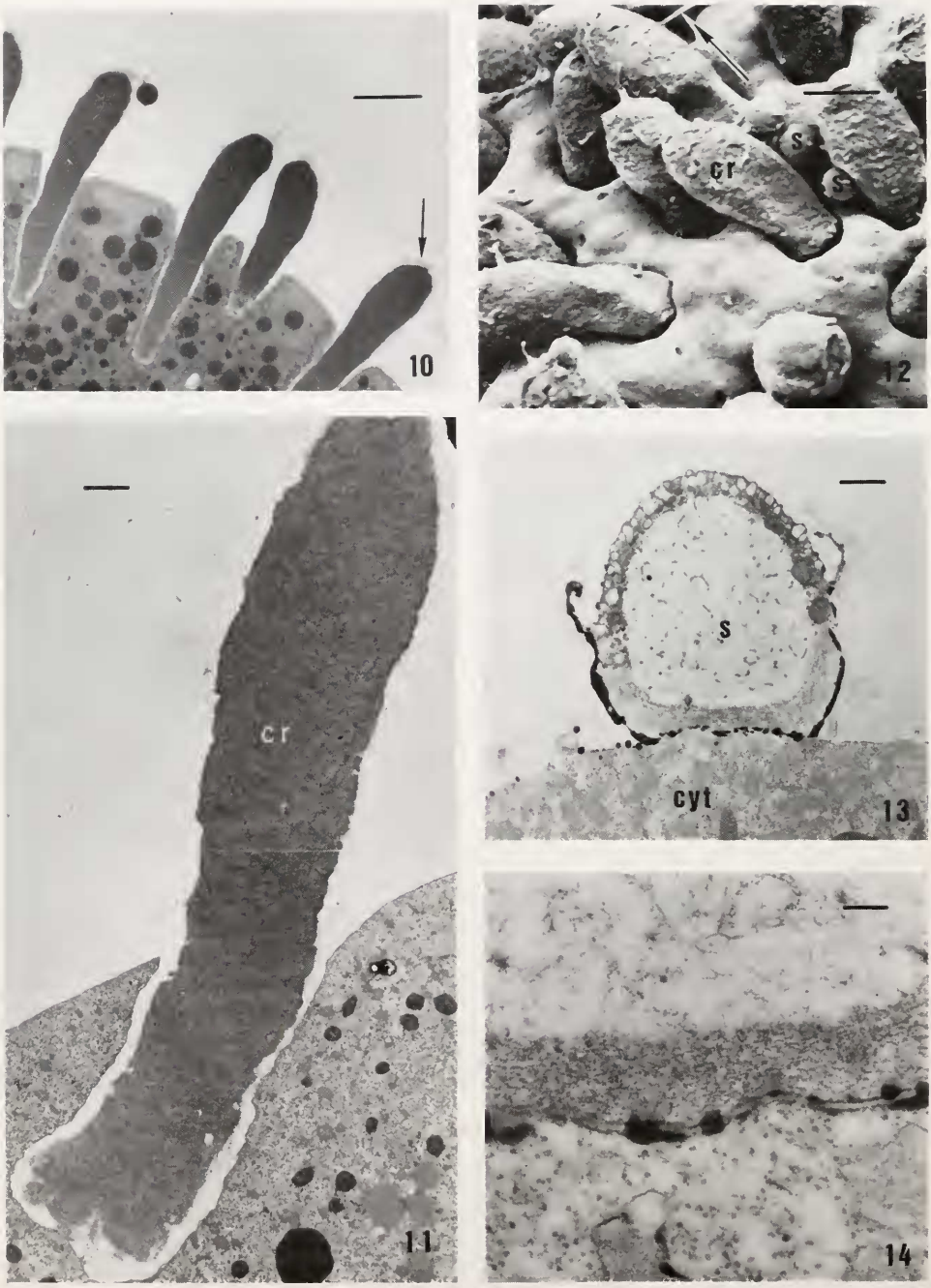


FIGURE 10. A thick plastic section of the corona stage. The arrows indicate the investment coat. Bar = 10 μm.

FIGURE 11. A transmission electron micrograph showing a cortical rod extending out of its crypt. Bar = 2 μm.

FIGURE 12. A scanning electron micrograph of an activated egg surface. Cortical rods (CR) are well out of their crypts. Occasional strands of investment coat extending between

Teleost eggs also undergo a massive cortical reaction (Yamamoto, 1939), retaining their extruded jellylike material beneath a vitelline membrane. This is in contrast to the penaeid eggs, which rapidly lose their dispersed cortical substances into the surrounding media.

In teleost eggs, the cortical reaction is initiated by sperm-egg contact and proceeds in a wavelike fashion from the point of sperm attachment (Yamamoto, 1939, 1961). The reaction in nereid eggs is also in direct response to sperm attachment (Costello, 1949), while in penaeid eggs, the reaction is stimulated by exposure to sea water and not by fertilization (Clark and Lynn, 1977). Although the cortical reaction in nereid eggs is initiated by sperm attachment, it is similar to the penaeid reaction in that it occurs simultaneously over the entire surface of the egg.

Another feature of penaeid ova is the decrease in the egg volume after the cortical reaction. A decrease in volume after the cortical reaction is also seen in the ova of the brook lamprey, *Entosphenus* sp. (Okkelberg, 1914), several echinoderms (Glaser, 1914; Eddy and Shapiro, 1976), and the anthozoan *Bunodosoma* sp. (Dewel and Clark, 1974). In *Bunodosoma* sp. eggs (Dewel and Clark, 1974), large regions of peripheral cytoplasm, interstitial to reacting and fusing cortical granules, are lost during egg activation (Dewel and Clark, 1974). Echinoderm eggs accommodate additional membrane from cortical vesicles by the elongation of surface microvilli (Eddy and Shapiro, 1976), while in penaeids, the excess membrane appears to be lost by a process of membrane vesiculation. The actual penaeid egg surface closely approximates the unactivated egg surface minus the crypt membrane and crypt apices surface area, though the volume lost during activation can be accounted for only partially, 50%, by initial rod volume. The mechanics of these reductions in cytoplasmic volume in penaeid eggs are poorly understood as are the functions of the cortical reaction itself.

Removal of supernumerary sperm from the surface of many animal ova is partially the result of a cortical reaction at the time of fertilization (Epel, 1975, for review). Many sperm on the surface of penaeid eggs are lifted off with the investment coat during the expulsion of the rods. Removal of supernumerary sperm from shrimp ova, however, does not appear to be the primary function of the cortical reaction, since the reaction is initiated by contact with sea water and not by sperm penetration. Two additional arguments against the cortical reaction's being a block to polyspermy are, first, the sperm are nonmotile and come into contact with the ova at the time of spawning (Clark *et al.*, 1974); and second, the large number of sperm attached to penaeid ova, as well as several sperm pronuclei within the oocytes (unpublished data), indicate that polyspermy is normal. This is not surprising since physiological polyspermy is known in many arthropods and is thought to be a prerequisite for successful fertilization (Austin, 1965, for review; Brown and Knouse, 1967; Hinsch, 1971).

Another possible function of the cortical reaction is the formation of a protective jelly layer. Such a layer may function as protection against an unstable environment (Allen, 1958; Schuel, 1978) as do the egg jelly or investment layers around

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the rods (arrow) and sperm(s) are seen associated with the rods and oolemma. Small depressions are noted in the surface of the oolemma. Bar = 10 μm.

FIGURE 13. Transmission electron micrograph of sperm(s) in close association with an egg. Egg cytoplasm (CYT). Bar = 1 μm.

FIGURE 14. A higher magnification showing the membranous association between the sperm and egg. Bar = 0.2 μm.

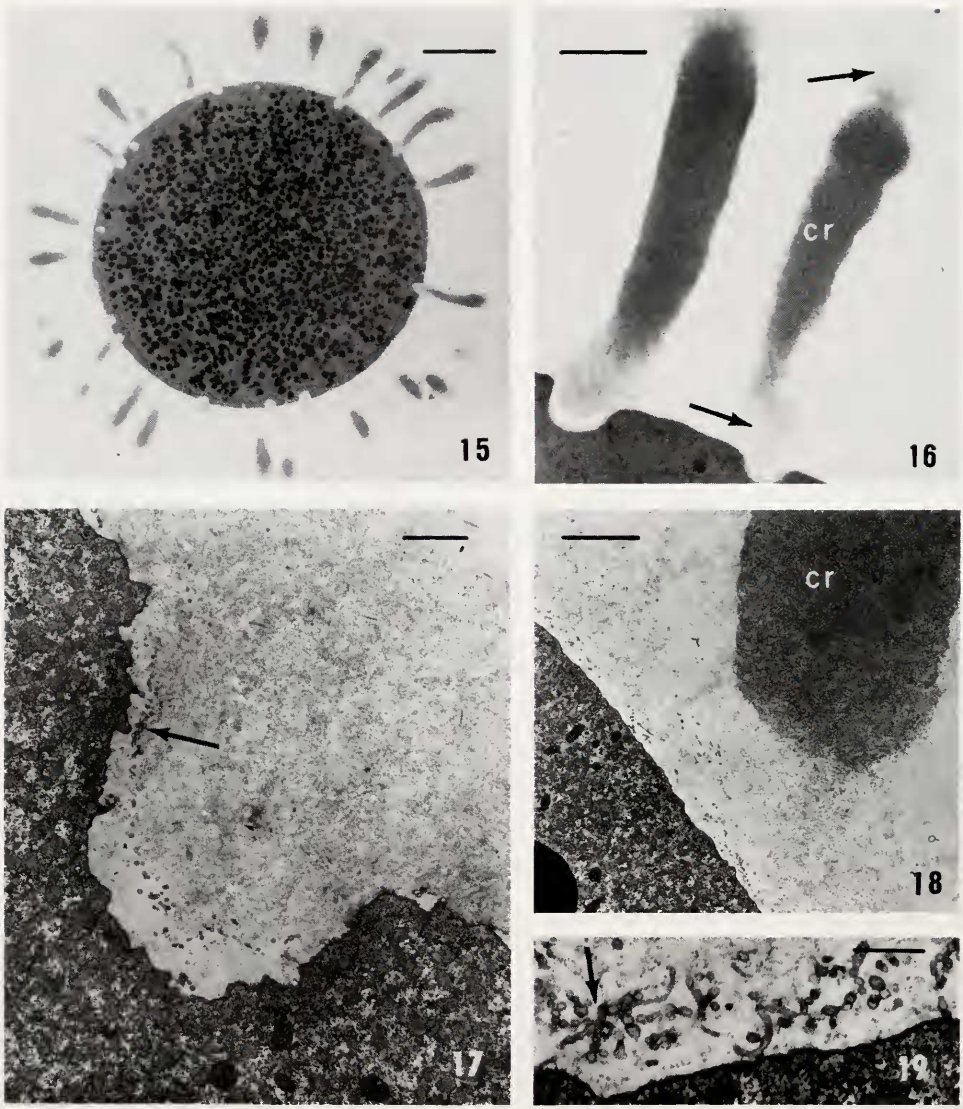

FIGURE 15. A light micrograph of an oocyte during the initial stages of cortical rod dissipation. Bar = 50 μm.

FIGURE 16. A higher magnification showing the cortical rods dissipating (arrows). Bar = 10 μm.

FIGURE 17. A transmission electron micrograph illustrating the base of a crypt, dissociated rod, and numerous small vesicular elements (arrow). Bar = 1 μm.

FIGURE 18. A fine structural view of cortical rod (cr) dissociation. Again, vesicular elements are noted along the oolemma. Bar = 2 μm.

FIGURE 19. A higher magnification of the membrane vesiculation (arrow) occurring directly adjacent to the oolemma. Bar = 0.2 μm.

many ova. Hudinaga (1942) reported that the dissipated cortical rod material is retained as a jelly coat until the second cleavage in *P. japonicus*. In ova of *P. aztecus* and *P. setiferus*, however, introduction of colloidal carbon to a slide of reacted eggs reveals no zone of jelly material after the formation of the hatching

membrane. At present, several lines of investigation are in progress to determine the function of the cortical reaction. We also have initiated experiments to increase understanding of the process of fertilization and the effect, if any, of the cortical reaction on sperm-egg interaction.

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Summary

The morphology of large cortical specializations (rods) located in the peripheral ooplasm is described in oocytes of the shrimp *Penaeus aztecus* and confirmed with *P. setiferus*. The rods, which are perpendicular to the oolemma, are "club"-shaped and approximately 39.9 μm long, with an apical diameter of 9.3 μm and a basal diameter of 4.6 μm. They are composed of numerous, tightly packed, fibrillar structures. Each cortical rod lies within a partially membrane-bound crypt and is separated from the external media by a thin investment coat. The investment coat lies directly adjacent to the oolemma and completely surrounds the egg. Upon exposure to sea water, the cortical rods begin to emerge from the crypts and appear to lift the investment coat off the egg surface. A corona is formed around the oocyte as the rods are expelled, but quickly dissipates. Extensive membrane vesiculation associated with cortical rod crypts is apparent at this time.

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